

Data Evaluation Report on the Acute Toxicity of the Nanosilver HeiQ AGS-20 to Freshwater Invertebrates - *Daphnia magna*

PMRA Submission Number {.....}

EPA MRID Number 49710101

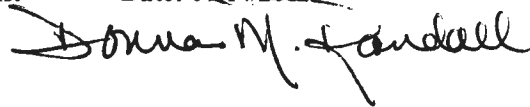
Data Requirement: PMRA Data Code:
EPA DP Barcode: 429650
OECD Data Point:
EPA Guideline:

Test material: HeiQ AGS-20 (Batch HQ-RCC-080311)
Common name: HeiQ AGS20
Chemical name: Sintered nanosilver-amorphous silica composite
IUPAC:
CAS name:
CAS No.:
Synonyms:

Purity (%): >99% HeiQ AGS-20;
17.4% silver nanoparticles

Reviewer(s) : Donna M. Randall, M.S., Senior Scientist/Ecotoxicologist
USEPA/OCSPP/OPP/AD/RASSB

Date: 07/14/2016



Reference/Submission No.: 973615

Company Code: [For PMRA]
Active Code: [For PMRA]
Use Site Category: [For PMRA]
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CITATION: Height, M. 2014. HeiQ AGS-20: Acute Toxicity to *Daphnia magna* in a 48-Hour Immobilization Test. Conducted and prepared by Harlan Laboratories Ltd., Itingen, Switzerland for HeiQ Materials AG, Bad Zurzach, Switzerland. Harlan Laboratories Study C51383. Study Experimental Dates: 11/13/2009-12/18/2009.

EXECUTIVE SUMMARY:

The 48-hour acute toxicity of HeiQ AGS-20, a sintered nanosilver-amorphous silica composite, to *Daphnia magna* was studied under static conditions. This study is classified as unacceptable for use in determining the quantitative toxicity to *D. magna* of HeiQ AGS-20 and its nanosilver component primarily due to the use of a water accommodated fraction (WAF) approach and lack of characterization of silver fractions (e.g., ionic, nano, remaining dissolved) to which the daphnids were exposed over the study duration, but there are also other issues that impacted the quality of the study.

Daphnids were exposed to a dilution control, a WAF of a stock solution of the end-use formulation product HeiQ AGS-20 (Batch HQ-RCC-080311), and five dilutions of the WAF. The geometric mean measured total silver concentrations of the WAF and the four least diluted WAF treatments were 25.1, 7.27, 2.79 and 0.480 µg/L. No analytical measurements of silver were made in the two most dilute treatment solutions (nominal 0.283 and 0.088 µg/L total silver). The control measured total silver concentration was below the limit of quantification (<0.500 µg/L total silver).

- No characterization of the nanosilver a.i. released from HeiQ AGS-20 and its major transformation products in the WAF was provided under test conditions. Only total silver (tAg) was measured in the test media; analytical methods did not include fractionating silver into its ionic, nano-sized, remaining “dissolved”, and non-dissolved components. That dissolution of a portion of the nanosilver in the test material is likely to have occurred is supported by statements in the study report regarding that the WAF was prepared in a manner “...to dissolve maximum amounts of the different compounds of the test item in the test water.”
- The use of a WAF for the purpose of having more dissolved compounds of HeiQ AGS-20 end use product dissolve does not meet protocols the Agency would use to establish the toxicity of a test material with

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nanomaterial components, for example see Section 5.2.1., bullet number 10 and Section 5.3, bullet number 9 in Coleman, Kennedy and Harmon (2015).

- The concentration of total silver in the water column was not stable as concentrations in three of the four treatments analyzed declined by more than half in one treatment and by more than 20% in two others, indicating a static test was not appropriate for this test material.
- The dilution spacing factor of 3.2 is larger than recommended and impacted the statistical model which could be used to determine the EC₅₀ and NOEC especially given a steep slope response was observed rather than a more flat concentration-effect relationship and that the effective dose spacing was actually 5.8 based on measured concentrations surrounding the EC₅₀ and NOEC/LOEC.

For the collective reasons discussed above, this study is classified as unacceptable for use in determining the quantitative toxicity to *D. magna* of HeiQ AGS-20 and its nanosilver component. Whatever the actual form of the test material, it appears more toxic than what is expected of dissolved silver alone based on ambient water quality criteria for silver. No paired daphnid acute test with silver nitrate was included in the study to document the toxicity to ionic and dissolved silver of the organisms used in this specific study. The national ambient water quality criteria maximum concentration at a water hardness of 250 mg CaCO₃/L, the water hardness in this study, for dissolved silver (dAg) is 15.6 µg dAg/L, which equates to an acute LC₅₀ or EC₅₀ of 31.1 µg/L dissolved silver versus the 1.16 µg/L total silver observed in this study. *Daphnia magna* was the most sensitive species to silver used to derive the silver ambient water quality criteria¹.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

- OECD Guideline for Testing of Chemicals, No. 202, *Daphnia* sp., Acute Immobilisation Test, 2004
- EU Commission Directive 92/69/EEC, C.2, Acute Toxicity for *Daphnia*, 1992.
- Commission Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), C.2, Acute Toxicity for *Daphnia*.
- OECD Series on Testing and Assessment No. 23. OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures.

COMPLIANCE:

Signed and dated No Data Confidentiality Claim, GLP Compliance and Quality Assurance Statements for the study were included and indicated that everything was conducted in compliance with the exception of pre-experiments (Appendix, p. 5 of report).

A. MATERIALS:

1. Test material

HeiQ AGS-20

Description:

Sintered nanosilver-amorphous silica composite powder

¹ <https://www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table>

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Lot No./Batch No. : Batch HQ-RCC-080311

Purity: >99% HeiQ AGS-20; % silver nanoparticles (Ag⁰) – not reported (label claim 19.3%)

Stability of compound under test conditions: Total silver (tAg) was monitored in exposure media, but no characterization of the size distribution or quantitative fractionation into nanosilver particle size content or distribution, and ionic silver concentration was made. The test material was not stable throughout the static test. The measured tAg concentration declined more than 20% in 3 of the 4 treatments analyzed (48-hour concentrations were 78.1%, 71.5%, 105%, and <54.3% of 0-hour concentrations in the WAF, 1:3.2, 1:10, and 1:32 dilution treatments, respectively. No analytical measurements were made in the 1:100 or 1:320 dilution treatments.

Table 1. Characterization of the nanosilver a.i. in HeiQ AGS-20 under test conditions

| Parameter | Values | Comments |
|----------------------------|-------------------------------|--|
| Shape | Not reported | Not analyzed under test conditions. |
| Size | 1 to 10 nm in diameter | Egger et al. 200); not analyzed under test conditions and not tested using the batch used in this study |
| Surface area | Measured but CBI | Both composite and metal component MRID 4889578, CBI |
| Size-to-surface area ratio | | |
| Zeta potential | Not measured | No information on the nanosilver a.i. using exposure media and test conditions were provided to the agency or cited. |
| Capping material | None | |
| Water solubility at 20 °C | 210.3 mg Ag/L (+2.46 mg Ag/L) | Pg 14 of study report. Temperature for solubility was not specified. |
| UV absorption | Measured but CBI | Measured in distilled water MRID 48878801, CBI |

CBI: Confidential business information

Storage conditions of test chemicals:

HeiQ AGS-20 (Batch HQ-RCC-080311) was stored at 25 °C by registrant and at room temperature (20 °C), away from direct sunlight by Harlan Laboratories. Stock solution stirring and equilibrium (5 days) was conducted in the dark at room temperature; resulting WAF stock solution was used immediately to prepare test media.

2. Test organism:

Species: *Daphnia magna* Straus, clone 5
Age at test initiation: ≤24 hours old and not first brood progeny
Source: In-house cultures

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B. STUDY DESIGN:

1. Experimental Conditions

a. **Range-finding Study:** A range finding study was reported as being conducted, but the results are not provided in the report.

b. Definitive Study:

Table 2. Experimental Parameters

| Parameter | Details | Remarks |
|---|--|--|
| <u>Acclimation</u> Period: Conditions: (same as test or not) Feeding: Health: (any mortality observed) | Not applicable: Same as test Not during test Mortality in cultures not reported, but stated as healthy and lacking in diseases. | Test dilution water is same as culture water and culture temperature same as test. Test control mortality within acceptable limits. |
| Duration of the test | 48 hours | |
| <u>Test conditions</u> Static/flow-through: Type of dilution system for flow-through method. Renewal rate for static renewal | Static Not applicable Not applicable | |
| Aeration, if any | No aeration during the test period. | |
| <u>Test vessel</u> Material: (glass/stainless steel) Size: Fill volume: | Glass beakers 100 mL 50 mL | |
| Biomass loading rate | 5 daphnia/50 mL = 1 daphnia/10 mL | |
| Source of dilution water | Reconstituted hard synthetic water | ISO 6341 method |
| <u>Dilution water parameters:</u> Hardness, mg/L as CaCO ₃ pH, IU Dissolved oxygen, mg/L Total Organic Carbon, mg/L Particulate matter, mg/L Metals, mg/L Pesticides, mg/L Chlorine, mg/L Alkalinity, mg/L as CaCO ₃ Conductivity, µS/cm COD (when testing cationic compounds) Intervals of water quality measurement | 250 7.9 8.6 Followed ISO 6341 to prepare reconstituted water therefore all of the following parameters should meet guideline standards for dilution water. -- 0.8 -- -- | |
| <u>Test solution water parameters:</u> pH, IU Dissolved oxygen, mg/L Temperature, °C | 7.9 to 8.0 8.4 to 8.7 20 (±0 °C) | |

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| Parameter | Details | Remarks |
|--|--|---|
| Intervals of water quality measurement | test initiation (0-hr) and termination (48-hr) | |
| <u>Stock solution concentration</u> Nominal: Measured: | 3.2 mg AGS-20/L 0.557 mg tAg/L – reviewer calculated based on assumption of 17.4% nanosilver a.i. content (nanosilver a.i. content of Batch HQ-RCC-080311 not stated in study report but based on ratio of conversion between silver and test material the reviewer calculated 17.4% silver content) WAF: 28.3 µg tAg/L | A water accommodated fraction method was used to prepare test solutions. Per page 17 of the study report, the stock solution was stirred for 96 hours (~750 rpm) at room temperature, then left to sit for 24 hours to allow particles to settle and then filtered through membrane filters (Schleicher & Schuell, Type NC20, pore size 0.20 µm [200 nm]). Only total silver (tAg) was measured, no fractionation or determination of ionic and particulate Ag was performed. |
| <u>Treatment concentrations</u> Nominal (corrected/uncorrected): Measured: | <u>Nominal tAg of WAF dilutions:</u> Control, WAF (28.3 µg tAg/L), and five WAF dilutions (1:3.2; 1:10; 1:32; 1:100; & 1:320) which correspond to 8.84, 2.83, 0.884, 0.283, and 0.088 µg tAg/L, respectively. Geometric mean of 0-hour and 48-hour measured tAg concentrations: <0.500 µg tAg/L (control), 25.1 (WAF), 7.27, 2.79, 0.480, n.m., n.m. µg tAg/L, respectively (n.m.: not measured) | Only total silver (tAg) was measured, no fractionation or determination of ionic and particulate Ag was performed. Used one-half the LOQ for the non-detect at 48 hours for the 1:32 WAF dilution treatment to calculate the geometric mean concentration. |
| Solvent (type and concentration, if used) | None | -- |
| <u>Number of organisms/replicate</u> Negative control: Positive control: Treatments: | 5 daphnia per replicate Not reported 5 daphnia per replicate | |
| <u>Number of replicates</u> Negative control: Positive control: Treatments: | 4 replicates Not reported 4 replicates per treatment group | |
| Lighting | 16 hours light:8 hours dark; 520 to 680 | 30-min transition period |

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| Parameter | Details | Remarks |
|--|--|--|
| Recovery of chemical Level of Quantitation Level of Detection Method recoveries % of nominal | 0.500 µg Ag/L Not reported 87% & 80% of spiked values (3.00 and 95.1 µg Ag/L, respectively); mean of 83% (n=4) Of the three WAF dilutions analyzed (1:3.2, 1:10, and 1:32) the 0-hour concentrations ranged from 96.1% to 104% of nominal | Method: Atomic Absorption Spectroscopy (AAS) |
| Variability of chemical in the test system | In the WAF and the three WAF dilutions analyzed (1:3.2, 1:10, and 1:32) all but the 1:10 dilution declined by over 20% of initial measured concentrations; the 1:10 at test termination was 105% of initial—see stability discussion | |
| Other parameters, if any | | |

2. Observations:

Table 3: Observations

| Parameter | Details | Remarks |
|--|---|---------|
| Parameters measured, including the sublethal effects | Survival, immobilization, behavior, morphology | |
| Observation intervals | <u>Lethal and sublethal effects</u> : test initiation, 24 & 48 hours <u>Ag analysis in test solutions</u> : test initiation and termination. <u>Water quality</u> : water temperature, pH, dissolved oxygen: test initiation and termination Appearance: test initiation, 24 and 48 hours. | |
| Were raw data included? | Yes | |
| Other observations, if any | | |

II. RESULTS AND DISCUSSION

A. MORTALITY/IMMOBILIZATION:

The number of organisms exposed at test initiation and the number and percent immobilized after 24 and 48 hours of exposure in a control, WAF and five dilutions of the WAF are summarized in Table 4. Control immobilization at test termination (0%) met guideline standards ($\leq 10\%$).

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Table 4: Effect of WAF of AGS-20 expressed as tAg^(a) on Immobility of *Daphnia magna*

| Treatment Group ^(a) | | No. of organisms at test initiation | Observation period | | | |
|--------------------------------|---|-------------------------------------|--------------------|---------------|--------------|---------------|
| Dilution | Geometric mean measured tAg µg/L [nominal] ^(a) | | 24 hr | | 48 hr | |
| | | | No. Immobile | % Immobilized | No. Immobile | % Immobilized |
| Control | <0.500 | 20 | 0 | 0 | 0 | 0 |
| 1:320 | na [0.088] | 20 | 0 | 0 | 0 | 0 |
| 1:100 | na [0.283] | 20 | 0 | 0 | 0 | 0 |
| 1:32 | 0.480 ^(c) [0.884] | 20 | 0 | 0 | 0 | 0 |
| 1:10 | 2.79 [2.83] | 20 | 20 | 100 | 20 | 100 |
| 1:3.2 | 7.27 [8.84] | 20 | 20 | 100 | 20 | 100 |
| WAF | 25.1 [--] | 20 | 20 | 100 | 20 | 100 |

na: not analyzed

^(a) Testing laboratory used measured tAg to extrapolate the HeiQ AGS-20 exposure concentration, based on measured tAg in stock. As only one concentration of HeiQ AGS-20 was used to prepare the stock and material was allowed to settle out and was filtered out using a 20 nm filter the dilutions of the WAF do not represent differing concentrations of HeiQ AGS-20 and are not expressed by the reviewer as such as compared to the report.

^(b) Reviewer calculated nominal concentrations of total silver (tAg) in dilutions of the WAF, based on measured tAg in the WAF stock at test initiation.

^(c) For calculating the geometric mean measured concentration, used one-half the LOQ for the non-detect analytical result at 48-hours.

B. SUB-LETHAL TOXICITY ENDPOINTS:

No sublethal effects were reported. Report does not specifically state none were observed but the guideline stated as followed calls for observing and reporting sublethal effects other than immobilization.

C. REPORTED STATISTICS: No statistical analysis was conducted because the response was all dead at the 1:10 dilution and no dead at the 1:32 dilution. The study authors calculated the 24-hr and 48-h EC₅₀ as the geometric mean of the 1:32 WAF dilution and 1:10 WAF dilution concentrations expressed as HeiQ AGS-20 concentrations (8.8 µg HeiQ AGS-20/L) and these treatment concentrations [3 & 16 µg HeiQ AGS-20/L] were also used as the confidence bounds for the EC₅₀ and as the NOAEC and LOAEC). The study authors converted measured tAg concentrations to HeiQ AGS-20 based on HeiQ AGS-20 batch being tested having a 17.4% silver content. Because the tAg concentration was non-detect at test termination in the 1:32 WAF dilution, the study authors used one-half the LOQ to calculate the concentration in the 1:32 dilution treatment. This resulted in an effective dose spacing factor of 5.8 between the treatment group with 100% mortality (1:10 WAF dilution and the treatment group (1:32 WAF dilution) with no mortality.

Reviewer agrees that the statistical approach of taking a geometric mean, or also called the binomial method by the Agency, is the method used when there is an all and none response. However, the guideline recommended dose-spacing of 2 limits the large uncertainty introduced into the EC₅₀ and NOEC/LOEC in such an all-or-none response. The 48-h EC₅₀ value based on measured tAg is 1.16 µg tAg/L, with confidence bounds of 0.480 µg tAg/L to 2.79 µg tAg/L which also correspond to the NOEC and LOEC, respectively.

D. CONCLUSIONS: This study is classified as unacceptable for use in determining the quantitative toxicity of HeiQ AGS-20 end-use product and it's nanosilver a.i. based on all the major issues identified that make the results unrepresentative – WAF approach to transform as much of the nanosilver a.i. to a dissolved form, lack of characterization of what organisms were actually exposed to in terms of nanosilver particulates, fractionated silver

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and ionic silver concentrations (example MRID 49725501), the instability of exposure concentrations over the duration of the study and therefore the static exposure versus at least static renewal, the enlarged dose spacing.

The 48-hr EC₅₀ for the WAF based on measured tAg is 1.16 µg tAg/L. This value is lower than the acute ambient water quality criteria for dissolved silver (dAg) of 15.5 µg/L at a water hardness of 250 mg CaCO₃/L, or the mean acute EC₅₀ value of 31.1 µg dAg/L at this water hardness¹. *Daphnia magna* was the most sensitive species to silver used to derive the silver ambient water quality criteria, so it is a representative measure. Therefore, the test material appears more toxic potentially than dissolved silver alone. No paired daphnid acute test with silver nitrate was included in the study to document the toxicity to ionic and dissolved silver of the organisms used in this specific study and laboratory, which means other sources of information on dissolved silver have to be used to inform the potential difference in toxicity.

E. REFERENCES:

- MRID 49725501 Kennedy, A.J., Hull, M.S., Bednar, A.J., Goss, J.D., Gunter, J.C., Bouldin, J.L., Vikesland, P.J., and Steevens, J.A. 2010. Fractionating nanosilver: importance for determining toxicity to aquatic test organisms. *Environmental Science and Technology* 44: 9571-9577 and Supporting Information pages S1-S7. Study Experimental Dates: not reported.
- Coleman, J., A. Kennedy, and A. Harmon. 2015. Nanoparticle Dispersion in Aqueous Media: SOP-T-1. Environmental Consequences of Nanotechnologies. U.S. Army Engineer Research and Development Center, U.S. Army Corps of Engineers. 25 pp. <http://el.erdc.usace.army.mil/elpubs/pdf/srel15-2.pdf>